

Report No. IITRI-L6023-7
(Quarterly Status Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

Contract No. NASr-22

National Aeronautics and
Space Administration
Washington, D.C.

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I. INTRODUCTION

Simulated Martian environment experiments are being conducted with Staphylococcus aureus. The effects of the following barometric pressures and carbon dioxide concentrations were studied:

- (1) Earth atmosphere at pressures of 10, 25, and 40 mb, with a diurnal temperature cycle
- (2) Carbon dioxide concentrations and pressures of 37% at 40 mb, 67% at 25 mb, and 100% at 10 mb, with a diurnal temperature cycle.

S. aureus grew in Earth atmospheres at all barometric pressures and in all concentrations of carbon dioxide. Growth was rapid and maximum populations were reached as early as 3 days with an 8-hr daily freeze and 7 days with a 20-hr daily freeze. Maximum populations were 100- to 1,000-fold higher than initial populations.

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Similar experiments were initiated with carbon dioxide concentrations of 37, 67, and 100% at 98 mb to determine the effect of the relative abundance of carbon dioxide on the growth of S. aureus.

Soil ecology experiments were initiated on the growth response of Bacillus cereus, Lactobacillus plantarum, Pseudomonas aeruginosa, Putrefactive Anaerobe (PA 3679), S. aureus, and Streptomyces albus in brunizemic and podzolic soils with 99% relative humidity and a constant temperature of 35°C or a diurnal temperature cycle with an 8-hr freeze. Cell populations as low as 10^2 to 10^3 cells per gram of soil were sufficient to permit growth of all bacterial species tested in both soil types except PA 3679.

Work was initiated on IITRI Proposal No. 66-492L (Revised) entitled "Design and Construction of Environmental Chambers." Technical discussions were held between IITRI Life Sciences and Engineering personnel. Construction of chambers will begin shortly.

II. EXPERIMENTAL PROCEDURES

S. albus stock culture was prepared by growing the organism on the surface of tryptone glucose extract agar (Difco) for 10 days at room temperature, after 10 days, abundant sporulation had occurred. The spores were harvested and washed in the usual manner with a 0.025 M phosphate buffer-0.1% Tween 80 solution at pH 7.0.

Report No. IITRI-L6023-5 described the stock culture preparation of B. cereus, P. aeruginosa, PA 3679, and S. aureus. Preparation of the L. plantarum stock culture was described in Report No. IITRI-L6023-6. All stock cell suspensions were stored at 4°C until used. B. cereus and PA 3679 spore suspensions were heat-shocked at 80°C for 10 min just before use.

Bacterial counts are reported as average counts of two plates from each of two or three tubes. Incubation was at 30 or 35°C for 24 or 48 hr, depending on the bacterial species.

III. RESULTS AND DISCUSSION

A. Simulated Martian Environment Studies

All tubes in these experiments contained 1 g of felsite/limonite soil, 1% organic medium, and 8.6 to 10.3% moisture. The tubes were flushed seven times with a particular gaseous atmosphere before pressure was established and the tubes were sealed. The balance of the 37 and 67% carbon dioxide atmospheres was argon and nitrogen. Diurnal freeze-thaw cycles of 8- and 20-hr freezes were used with each atmosphere. Control tubes were prepared in a similar manner, except they were flushed with Earth atmosphere and sealed at 98 mb. One-half of the control tubes were incubated at 35°C, and one-half received a diurnal temperature cycle with an 8-hr freeze. Tubes

from both control groups were sampled at 3 and at 7 days. Experimental tubes were sampled immediately and at 1, 3, 7, 28, and 56 days.

The experimental results are complete only through 7 or 28 days and are presented in Figures 1 through 3. A comparison of these figures indicated that Earth atmosphere at barometric pressures of 10, 25, and 40 mb with a 8-hr daily freeze had no effect on the growth of S. aureus. Similar maximum populations were reached in these tubes between 1 and 7 days.

Carbon dioxide concentrations of 37, 67, and 100% at respective pressures of 40, 25, and 10 mb with an 8-hr daily freeze cycle did affect the growth of S. aureus. Maximum populations in these tubes were generally 10-fold lower than the populations reached in tubes with Earth atmosphere at similar barometric pressures.

Extending the daily freeze to 20 hr delayed S. aureus growth in the tubes with Earth atmosphere at 10, 25, and 40 mb. Although growth was delayed between 1 to 3 days, the maximal populations reached were similar to populations in tubes receiving Earth atmosphere at the same pressures and a daily 8-hr freeze.

The growth of S. aureus in the tubes that received 37, 67, and 100% carbon dioxide concentrations and a 20-hr daily freeze was delayed from 3 to 7 days. There are indications that growth, although delayed, will achieve population maxima similar to the maxima recorded for the tubes with the same carbon dioxide concentrations and an 8-hr freeze.

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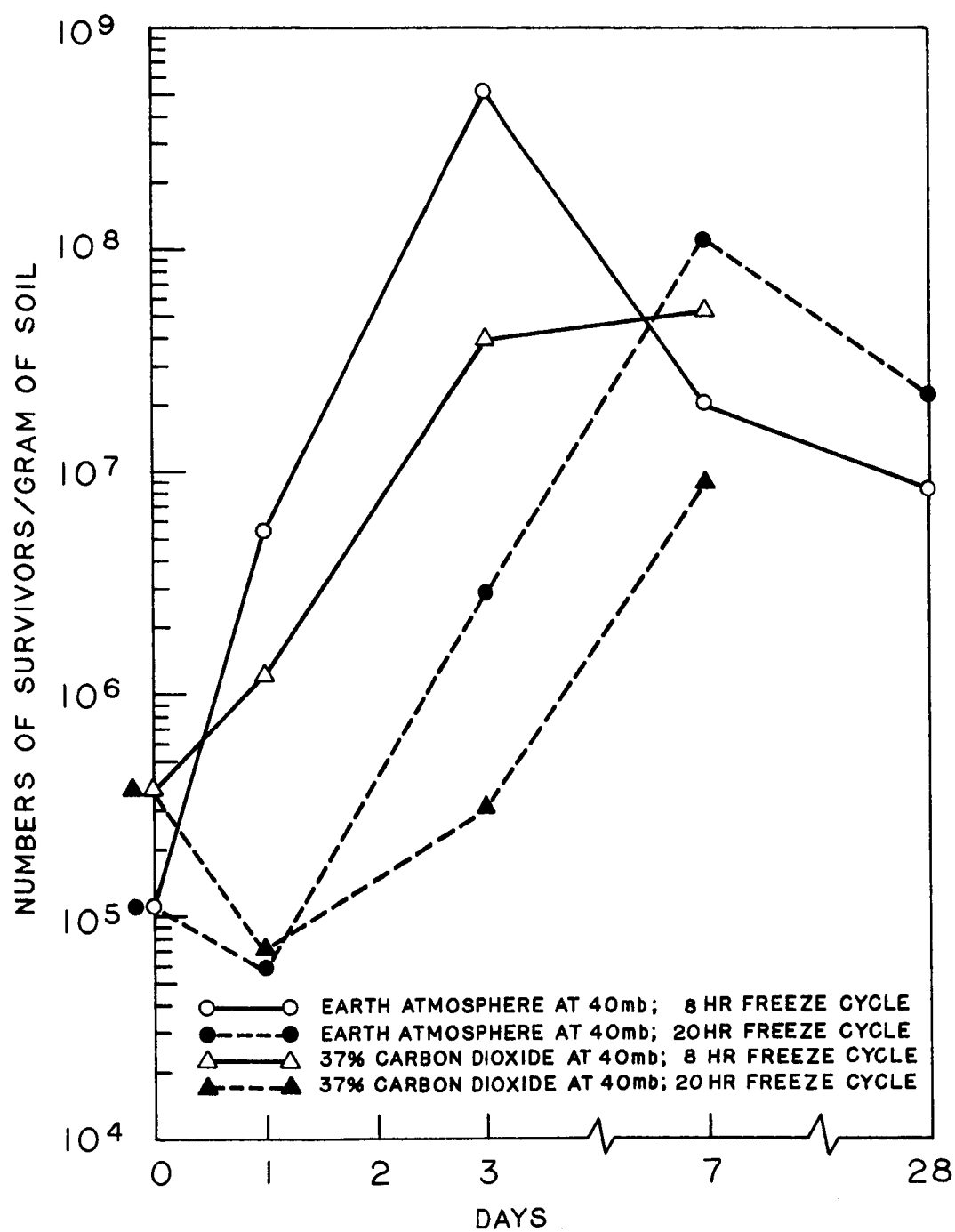


FIG.1 THE EFFECT OF DIFFERENT GASEOUS ATMOSPHERES AT 40mb PRESSURE WITH DAILY FREEZING AND THAWING ON SURVIVAL OF STAPHYLOCOCCUS AUREUS.

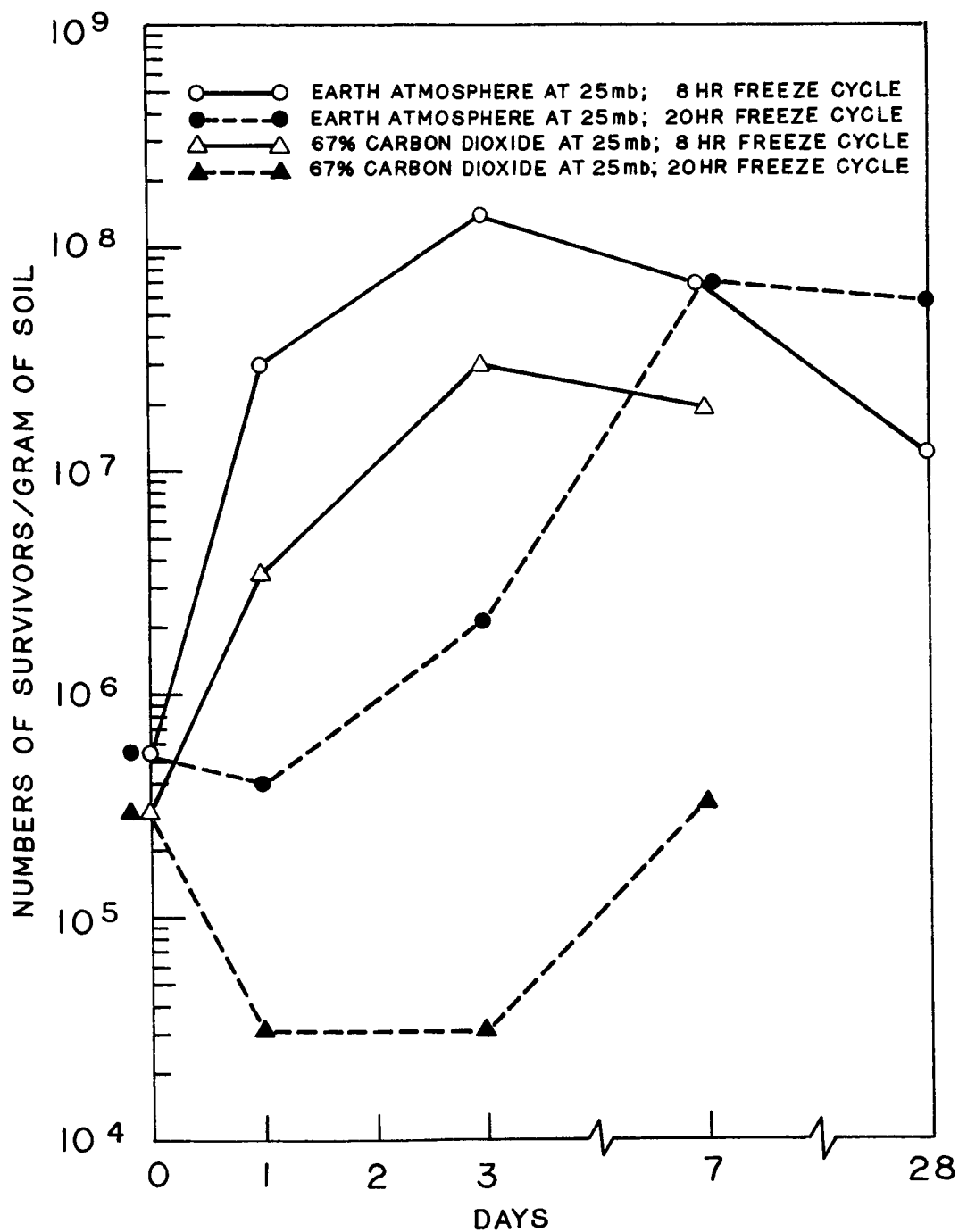


FIG. 2 THE EFFECT OF DIFFERENT GASEOUS ATMOSPHERES AT 25mb PRESSURE WITH DAILY FREEZING AND THAWING ON SURVIVAL OF STAPHYLOCOCCUS AUREUS.

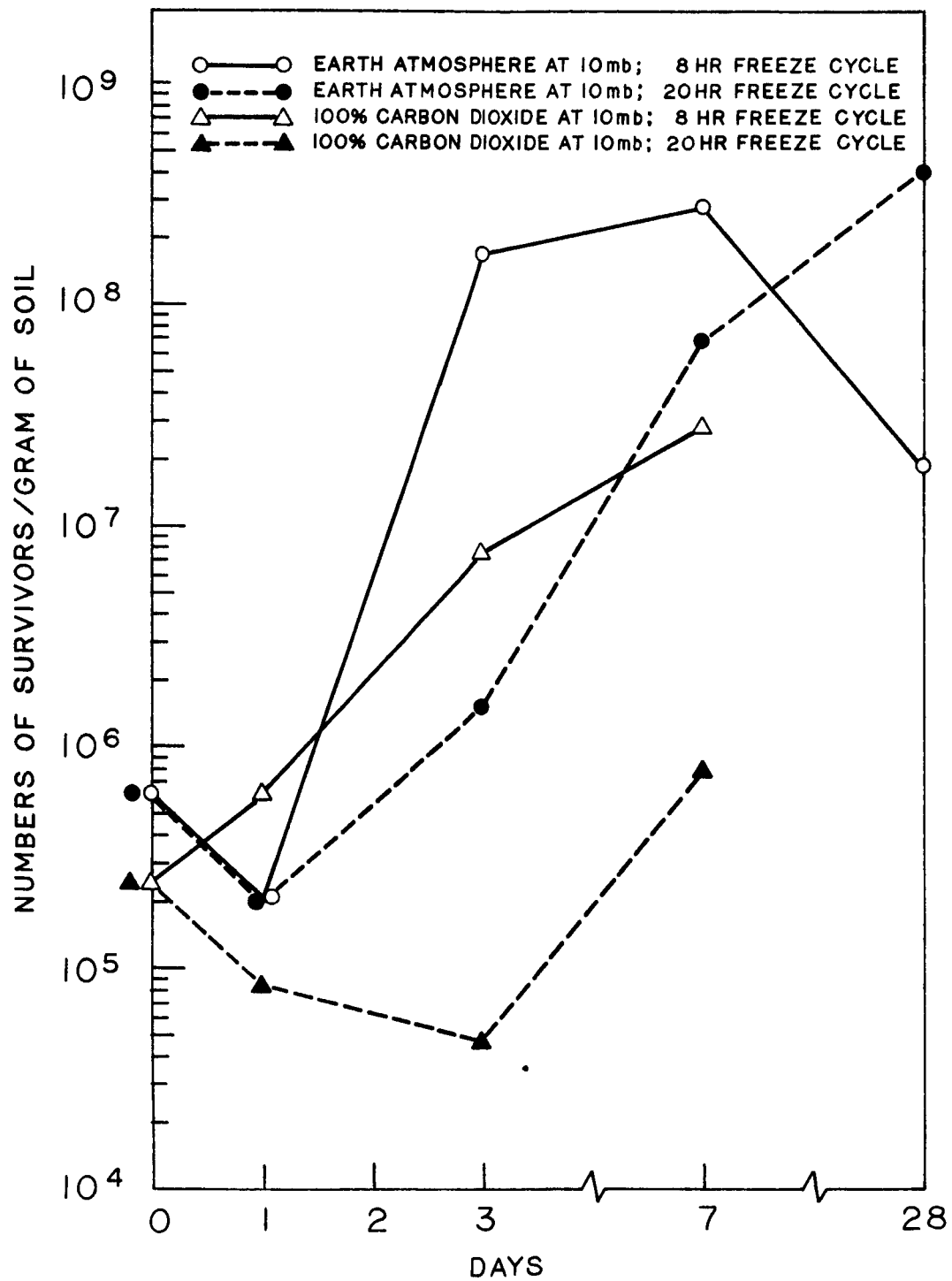


FIG. 3 THE EFFECT OF DIFFERENT GASEOUS ATMOSPHERES AT 10mb PRESSURE WITH DAILY FREEZING AND THAWING ON SURVIVAL OF STAPHYLOCOCCUS AUREUS.

There are several factors responsible for the rapid growth and survival of S. aureus in these severe environments. These are (1) resistance to daily freezing and thawing, (2) ability to grow in the absence of oxygen, (3) ability to tolerate high carbon dioxide concentrations, (4) relatively short generation time, and (5) low water activity (a_w) requirement for growth. It is the possession of these factors that enables S. aureus to respond in a very positive manner to extreme environments. These same factors, in turn, can be used as guidelines when considering the ability of other organisms to survive in similar severe environments. An organism's resistance to freezing, thawing, elevated carbon dioxide concentrations, and lack of a requirement for oxygen allow survival, while the short generation time and low a_w requirement permit the organism to multiply at lower temperatures during the freezing phase and to undergo earlier cellular multiplication during the thawing phase.

B. Soil Ecology Studies

Two soil types were collected in Kane County, Illinois. Collection and characterization of these soils were done with the cooperation of the U.S. Department of Agriculture soil scientists K. C. Hinckley and R. Montgomery from the Soil Conservation Service at Batavia, Illinois.

The brunizemic soil is of the Saybrook silt loam variety; it is a dark brown, moderate-to-fine granular structure, friable, and neutral soil. This soil, developed under native grasses, was collected from well-drained, gently sloping uplands. The podzolic soil is of the Dodge silt loam variety; it is a gray-brown, weak-to-fine granular structure, very friable, and medium-acid soil. This soil, developed under native hardwood vegetation, also was collected from well-drained, gently sloping uplands. Laboratory pHs of soil pastes prepared from the soils (one part soil to one part water) were 7.1 and 5.7 for the brunizemic and podzolic soils, respectively.

All tubes in the experiments contained 1 g of previously sterilized soil, sufficient water to establish an a_w of 0.99, and an Earth atmosphere of 1013 mb. The tubes were inoculated with particular organisms at predetermined cell populations and sealed. Half the tubes were incubated at 35°C, and half received a diurnal freeze-thaw cycle with an 8-hr freeze. Tubes were sampled immediately and at 7, 28, and 56 days.

Data on the growth of B. cereus, PA 3679, L. plantarum, P. aeruginosa, S. aureus, and S. albus in the brunizemic and podzolic soils are given in Table 1. The experiments are not complete, but certain trends can be presented at this time.

Table 1

EFFECT OF SOIL TYPE ON GROWTH RESPONSE OF BACTERIA^a

Organism	Number of Cells Inoculated/g of Soil	% Viable Cells Recovered		Ability to Establish an Ecological Niche ^b			
		After Inoculation Brunizemic Soil	Podzolic Soil	Brunizemic Soil		Podzolic Soil	
				Constantc	Diurnald	Constantc	Diurnald
<u>B. cereus</u>	4	52	105	+	+	+	0
	10 ³	25	86	+	+	+	0
	10 ²	43	67	+	+	+	0
PA 3679	4	42	101	-	-	-	-
	10 ³	43	83	-	-	-	-
	10 ²	43	220	-	-	-	-
	10						
<u>L. plantarum</u>	5	92	13	+	+	+	+
	10 ⁴	77	9	+	+	+	+
	10 ³	82	12	+	+	+	+
	10						
<u>P. aeruginosa</u>	4	65	3	+	+	+	-
	10 ³	40	5	+	+	-	-
	10 ²	67	7	+	-	-	-
	10						
<u>S. aureus</u>	5	37	54	+	+	+	+
	10 ⁴	67	42	+	+	+	+
	10 ³	78	54	+	+	+	+
	10						
<u>S. albus</u>	5	100	100	+	+	+	-
	10 ⁴	49	44	+	+	+	-
	10 ³	85	41	+	+	+	-
	10						

^aThese results are based upon experiments in progress (7 and 28 days).^b+ indicates increase, - indicates decrease, and 0 indicates no change in numbers as compared to initial count.^cIncubation at constant 35°C.^dIncubation with diurnal 8-hr freeze (-65°C) and 16-hr thaw (30°C) cycle.

The percent recovery of viable organisms after inoculation was generally higher from the brunizemic than the podzolic soil. On the assumption that other conditions were equal, the poorer recovery was attributed to the acid pH of the podzolic soil. Conversely, the acid pH of the podzolic soil prevented or delayed germination of B. cereus and PA 3679 spores and was reflected in the high percent recovery of these organisms from podzolic soil.

The incubation temperature did not adversely affect survival or growth of organisms in the brunizemic soil, but did in the podzolic soil. In the podzolic soil viability of many of the organisms decreased after 7 days. This result is believed to have been caused by the acid pH of the podzolic soil. The organic content of a soil would also affect a cell's recovery after inoculation and its survival potential. Although organic analyses of the soils have not been completed, the podzolic soils usually have a lower organic content than brunizemic soils.

Table 1 shows that 10^2 to 10^3 viable cells of B. cereus, L. plantarum, S. aureus, and S. albus per gram of soil can grow in brunizemic and podzolic soils at constant 35°C and in brunizemic soil with a diurnal temperature cycle. Also, although 10^2 cells of P. aeruginosa per gram of soil are sufficient for growth in brunizemic soil at constant 35°C, 10^3 cells/g are required for growth with a diurnal temperature cycle and 10^4 cells/g are required for growth in podzolic soil at constant 35°C.

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These studies are continuing, and complete data and evaluation of the experimental data will be presented after completion of the 56-day test period.

IV. SUMMARY

Growth of S. aureus was not inhibited in Earth atmosphere at barometric pressures of 10, 25, or 40 mb. Growth was rapid and abundant.

Carbon dioxide concentrations of 37, 67, and 100% at pressures of 40, 25, and 10 mb, respectively, did not inhibit the growth of S. aureus.

Maximum populations of S. aureus were 100- to 1,000-fold higher than initial populations.

Brunizemic and podzolic soils tested were adequate to support the growth of B. cereus, L. plantarum, P. aeruginosa, PA 3679, S. aureus, and S. albus. An 8-hr daily freeze did not affect the growth of these organisms in the brunizemic soil, but did decrease or inhibit growth in the podzolic soil.

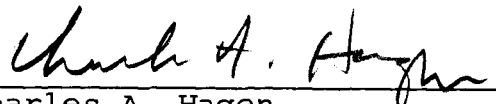
V. PERSONNEL AND RECORDS

The experiments were planned with the counsel of Dr. E. J. Hawrylewicz and the technical assistance of Mr. Bruce Anderson, Miss Marjorie Ewing, and Miss Vivian Tolkacz.

Experimental data are recorded in IITRI Logbooks C17091, C17092, C17094, C17096, C17097, and C17260.

Respectfully submitted,

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